Determination of Surface Tension of Packings for High-Performance Liquid Chromatography

Sorption or proteins to solids is a function of the solvent (γ_{LV}), protein (γ_{pv}), and solid (γ_{sv}) surface tensions. γ_{LV} values are measured routinely and contact angle approaches have yielded reliable values of γ_{pv}. However, only recently have methods for direct measure of surface tensions of small particles become available. We have determined the surface tensions of a number of surface-modified silicas with the most straightforward of these methods—the sedimentation volume technique. Surface tensions of alkylsilicas were in 32–39 mJ/m^2 range, as was an aryl-substituted support. Diol, diether, and polyamidosilicaceous-hydrophobic-interaction supports were more polar with γ_{sv} between 47 and 53 mJ/m^2. Since the surface tensions of most proteins were ~65–70 mJ/m^2, their sorption to all of these packings is predicted on the basis of a free energy model which considers interfacial tensions. However, much larger reductions in mobile phase surface tension are required to desorb proteins from the apolar packings than from the higher energy materials.

The adsorption of proteins to latex particles (*1*), their detachment from phenyl sepharose (*2*), and their elution in reversed-phase high-performance liquid chromatography (*3*) were shown to be related to the grand canonical function which we here simply refer to as free energy of interaction which is given by the expression

$$\Delta F_{smp} = \gamma_{sp} - \gamma_{sm} - \gamma_{mp} \quad (1)$$

where γ is the interfacial tension and the subscripts s, m, and p represent sorbent, mobile phase, and protein, respectively. Equation 1 is based historically on the paper by Hamaker (*4*) in which the attractive interaction between colloidal particles was related to the molecular properties of the substrate, on subsequent works (*5*, *6*) in which theoretical examination of the mathematical properties of Hamaker interaction constants suggested that repulsion could, under certain conditions, occur between dissimilar particles, and, finally, on thermodynamic treatments that related Hamaker interaction constants and surface tension (*7*). This literature is summarized well in ref 8. The interfacial tensions may be calculated through an equation of state (*9*) from the individual surface tensions. Measurement of liquid surface tension is straightforward and determination of protein surface tension may be deduced from measurement of contact angles of liquids of known surface tension on thick protein layers (*10*). Until recently, however, surface tensions of solid adsorbents were approximated by analogy to the substituted reactive groups such as phenol in phenylsepharose or octadecane in reversed-phase packings. In this paper, we describe a simple method for measuring the surface tensions of solids such as HPLC column packings. The method is based on the observation that solid particles will form a column of least tightly packed particles when the surface tension of the particles equals that of the liquid in which they are suspended (*11*, *12*). The underlying cause for this observation is that, under the condition described, van der Waals attractive forces will be minimal. In the case of nonpolar liquids, a minimum in sedimentation volume (V_{sed}) will be observed, whereas in polar liquids a maximum in V_{sed} will occur (*11*, *13*). It should be noted that the position of the extrema (minimum or maximum) always occurs at the same surface tension of suspending liquid, regardless of the type of extrema. A method based upon floatability for evaluating particle surface energy characteristics has been proposed recently (*14*).

Table I. Surface Tensions of Propanol–Water Mixtures at 25 °C

1-propanol (vol %)	1.5	2.5	5	10	15	20	30
surface tension	57	53	48	40	34	30	38

EXPERIMENTAL SECTION

Suspending Liquids. There are several ways in which experiments of this type may be performed. In the present study we have elected to employ a fixed mass of particles which are then suspended in a series of liquids having different surface tensions. We used binary mixtures of liquids one of which had a high surface tension and the other a low surface tension. By appropriate variation of the volume percentages of the two liquids, it was possible to produce a wide range of suspending liquid surface tensions. Liquids of different surface tensions were prepared by combining the required proportions of analytical grade propanol (Fisher) and deionized water. These liquids were selected because they span the desired range of surface tensions and because they were widely used in HPLC of proteins. Surface tensions were measured with a Wilhemy balance technique. It is important to determine liquid surface tensions immediately before use on each occasion. Table I may be consulted for guidance in preparing suspension liquids.

Column Packings. Nine derivatized silicas (5 μm), with pores of either 10 or 30 nm diameter, were prepared and supplied by Supelco, Inc. (Bellefonte, PA). The polyamidopropyl packing was purchased from SynChrom, Inc. (Linden, IN). Packings were washed by slurrying them sequentially in hexane, methanol, and methanol/water and removing the solvents by filtration through an 0.45-μm membrane filter. The packing was dried in a vacuum oven at 60 °C after the last wash.

Sedimentation. Two hundred milligrams (±0.1 mg) of packing was weighed into 2-mL polyethylene microcentrifuge tubes. Five hundred microliters of suspending liquid with known surface tension was added. The particles were dispersed in the liquid by vibration allowing particles to settle. This process was repeated several times briefly to assure that trapped air was displaced.

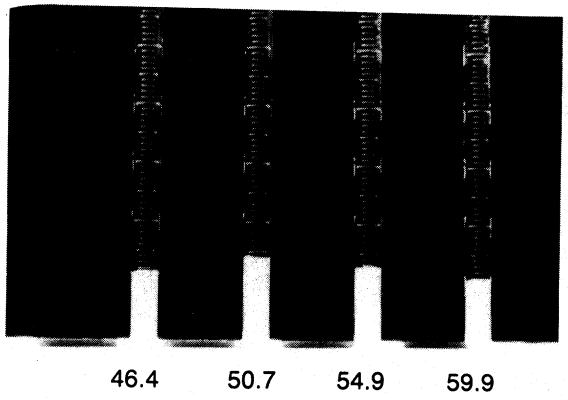

Figure 1. Sedimentation of diether LC packing. Numbers indicate surface tensions of suspending liquid.

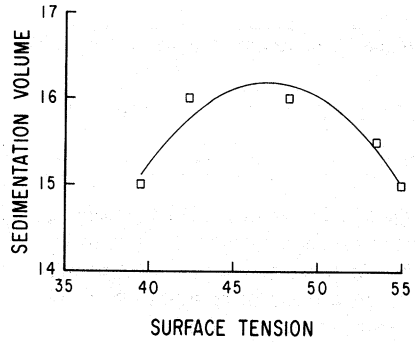

Figure 2. Plot of sedimentation volume of diol LC packing against surface tension of suspending liquid.

Finally the suspension was transferred quantitatively to graduated hematocrit tubes (Becton-Dickenson, Rutherford, NJ) with a Pastuer pipet. Hundred microliter portions of liquid were used to rinse the centrifuge tubes and pipets several times to ensure quantitative transfer of the particles. The hematocrit tubes were then filled to capacity (1 mL). The tubes were sealed with plastic wrap and inverted to assure that the particles were dispersed homogeneously. The particles were allowed to settle under the influence of gravity until no change in bed height was observed (~24 h). At least three determinations of the position of sedimentation maximum were made.

RESULTS AND DISCUSSION

When derivatized silica particles are dispersed in liquids of different surface tensions and permitted to settle, the volumes of the settled beds are not equal as shown in Figure 1. Figure 2 illustrates a plot of sedimentation volume against liquid surface tension of the suspending liquids. The maxima of three replicate determinations agreed to within ±1 mJ/m^2. It has been shown elsewhere (*11*) that the surface tension of the liquid at the position of maximum is equal to the surface of the solid particles. It was demonstrated in that work that similar positions were obtained when different liquids were employed to cover the same surface tension range.

Surface tensions (γ_{sv}) for various silica-based HPLC packings were determined and are reported in Table II. As a group, these packings would be classified as "low-energy" solids when compared to high-energy materials such as ice (106 mJ/m^2), glass (700 mJ/m^2), or platinum (1800 mJ/m^2) (*15*). Reported (*16*) γ_{sv} for other low-energy solids include Nylon-6.6 (44 mJ/m^2), polystyrene (32 mJ/m^2), Teflon (19.6 mJ/m^2), and siliconized glass (18.5 mJ/m^2). Surface tensions of the packings are higher than those of corresponding liquids. The range of values for C_6–C_{16} alkanes, for example, is 18–28 mJ/m^2 (*17*) and for polyethylene glycols, 42–46 mJ/m^2 (*18*). The higher polarity of the packings probably reflects the presence of unreacted silanol groups and the polar character of the siloxy bonds. The effects of silanols, carbon number,

Table II. Surface Tensions of Siliceous HPLC Packings

packing	surface tension, mJ/m^2	packing	surface tension, mJ/m^2
n-hexyl	31.7	diether DB[b]	39.1
tert–butylphenyl	35.0		
n-butyl	35.5	*tert*-butyl	39.2
n–butyl (300 Å)[a]	37.8	diol	47.0
n–butyl DB[b]	41.1	diether	51.9
n-octadecyl	36.6	polyamidopropyl	53.0

[a] 300 Å pore diameter, all others 100 Å. [b] Designated as deactivated for basic compounds.

Table III. Protein Adsorption on Derivatized Silicas

protein	octadecyl- γ_{mv}	octadecyl- γ_{sv}	octadecyl- ΔF	diether- γ_{sv}	diether- ΔF
BSA[a]	73	36.6	−4.2	51.9	−2.5
OVAL[b]	73	36.6	−5.5	51.9	−2.7
BSA	60	36.6	+8.2	51.9	+2.9
OVAL	60	36.6	+7.6	51.9	+2.7
BSAD[c]	60	36.6	−16	51.9	−6

[a] Surface tension of BSA taken as 70 mJ/m^2 (*2*). [b] Surface tension of OVAL taken as 69 mJ/m^2 (*2*). [c] Surface tension of denatured BSA taken as 36 mJ/m^2 (*3*).

and orientation of bonded group on the surface characteristics of silanized glass capillaries have been discussed in detail elsewhere (*19*). The data for the *n*-butylsiloxy silicas indicate that pore diameter effects on the surface tensions of particles are negligible. However, a treatment used by the manufacturer to reduce adsorption of basic compounds altered surface tensions measurably. Since the treatment is proprietary, we can only present the observation without discussion.

The mean surface tension for the six hydrocarbonaceous silicas (DB omitted) was 36 mJ/m^2 with a mean deviation of 1.9 suggesting that chain length or branching of alkyl groups has little influence on surface properties of these materials. The insensitivity of protein recovery to chain length of alkyl groups on derivatized silicas has been reported (*20*) and may be explained, in part, by the present observations. While the surface tension of the arylsilica falls within this group, this support may demonstrate different selectivities toward protein sorption because of π-electron interactions. The differences between these materials and the diol, diether, and amidopropyl packings are significant as shown in Table II. These data demonstrate the potential of the sedimentation volume technique for providing a scale by which to classify packings for chromatography. Such classification could evolve into a predictive tool similar to solubility parameter approaches for liquid extraction. Compilations of measurements on many packings would be needed for general utility.

It is beyond the scope of this report to justify the conceptualization of eq 1 and its implication in biological systems as these have been reviewed extensively (*7*, *21–24*) elsewhere. However, it is pertinent to discuss some generalizations as applied to chromatography from consideration of van der Waals attraction/repulsion concepts as shown in eq 1 and of measured surface tensions of column packings (γ_{sv}), proteins (γ_{pv}), and mobile phases (γ_{mv}). Values of ΔF_{smp} for two modified silicas are given in Table III for bovine serum albumin (BSA) and ovalbumin (OVAL) with two hypothetical mobile phases. When ΔF_{smp} has a negative value, protein sorption is predicted whereas positive values predict no sorption. The higher γ_{mv} is close to that of water containing ~0.1 mM NaCl or Na_2SO_4. With such mobile phases sorption of proteins is favored on all packings although binding affinity is predicted to be reduced greatly at higher γ_{sv} values.

Packings with such surface tensions are used commonly for size-exclusion chromatography or "hydrophobic interaction" chromatography (HIC) in which it is desirable to reduce nonspecific sorption to the matrix. The latter are similar chemically to the former but lightly alkylated. Under conditions where the protein is in a high γ_{mv} environment, the energy of interaction becomes similar for the proteins as γ_{sv} increases but is always negative. However, when the surface tension of the moble phase is reduced until it has a value less than that of the protein, ΔF_{smp} becomes positive. Ideally then, determination of molecular size should be conducted with such mobile phases to prevent sorption.

Further addition of salt increases γ_{mv} and promotes sorption. For the lightly alkylated supports, then it is predicted that protein retention can be manipulated by varying salt concentration. We may relate ΔF_{smp} to a common measure of chromatographic retention, k'

$$k' = \frac{V_R - V_m}{V_m} = K(V_s/V_m) = e^{-\Delta F/RT}(V_s/V_m) \quad (2)$$

where V_s/V_m is the phase ratio and K is the distribution coefficient and V_R is the protein retention volume. A strategy, then, for HIC is to introduce the protein mixture with high surface tension (salt) mobile phase and then reduce surface tension, eluting each protein as γ_{mv} passes that of the protein. Reduction of surface tension may be accomplished by reducing salt concentration or by addition of a modifier such as ethylene glycol. The adsorption concept does not evoke concepts of repulsion by the solvent but concepts of van der Waals attraction/repulsion of the protein and the surface that are accounted for by thermodynamic considerations. It should be noted, considering the amphiphilic nature of proteins, that the van der Waals attraction between an apolar species and a polar one in water has been shown to be significant (*2*, *25*).

As the surface alkyl group density is increased further or is the only surface modification, γ_{sv} is substantially lower and ΔF becomes much more negative. Under these conditions it is known that mobile phases containing very large proportions of organic additive in buffer are required to desorb proteins (*3*). Such proportions of organic modifier can alter the secondary structure of many proteins as observed by spectroscopic methods (*26*) and as evidenced by lower measured surface tensions (*3*). Solvent denatured BSA, for example, has a surface tension of $\sim$36 mJ/m^2 whereas the value of native protein is $\sim$70 in buffer. As seen in Table III, the solvent denatured BSA is predicted to have much greater attraction to a reversed phase column than the native protein as is indeed noted experimentally (*3*).

The sedimentation volume method provides a relatively simple means for characterizing the surface tension (γ_{sv}) or HPLC column packings. The relationship between γ_{sv} and protein sorption can be predicted on the basis of a knowledge of the surface tensions of the three interacting phases. Experimental conditions required to achieve elution of proteins from HPLC matrices can be explained also by a consideration of the magnitude and sign of the free energy of interaction computed from a knowledge of the surface tension of interacting protein and the surface tension of packing as determined by suspending packing particles in liquids of known surface tension.

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